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An Experimental Study on Congenital Biliary Dilatation

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Introduction

Congenital biliary dilatation was first described by Vater[24] in 1723 and reported as a disease by Douglas[6] in 1852. Babbitt[3,4] proposed a theory that biliary dilatation is caused by weakness of the choledochal wall owing to refleux of pancreatic juice into the bile duct, possibly due to an anomalous junction of the pancreaticobiliary ductal system. On the other hand, stenosis of the distal choledochushas been recognized to be closely related to biliary dilatation. Ando et al[2]. and Ito et al[9] are of opinion that biliary dilatation does not occur in the presence of an anomalous junction of the pancreaticobiliary ductal system in all patients.

In the present study[17], we investigated congenital biliary dilatation in 45 patients and found that the disease could be classified into two types, i. e., cystic type and cylindrical-fusiform type and that congenital biliary dilatation due to an anomalous junction of the pancreaticobiliary ductal system was of cylindrical-fusiform type in most patients. In this experimental study, the effect of stenosis of the distal choledochus by ligation and dissection was investigated to elucidate the etiology of the congenital biliary dilatation.

Materials and Methods

1. Animals (Table I)

One hundred and twenty-three Wistar rats were divided into 5 groups regardless of sex : 14 in Group I (control) ; 9 in Group II (single ligation of the choledochus) ; 70 in Group III (short-term ofservation after ligation and dissection of the choledochus) ; 15 in Group IV (long-term observation after ligation and dissection of the choledochus) ; 15 in Group V (cyst-duodenostomy). The adult rats were fasted from the day before operation and young rats aged 3-6 weeks were fasted from the morning of the operation and operated on in the afternoon.

The fasting time was 24 hours for the abults and 6 hours for the young.

2. Operation method

The animals anestherized with 0.025-0.05 ml/kg of i.p. Nembutal® were fixed on the operation

Key words: Congenital biliary dilatation, Ligation of the bile duct, Proliferation of bile ductuli, Choledochalcyst duodenostomy
索引語：先天性胆道拡張症，総胆管結紮，胆管増生，嚢腫十二指腸吻合
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Group	Rat No.	Body weight (g)	Shape of extrahepatic bile duct c: cystic type f: fusiform type	Diameter of the extrahepatic bile duct	Date of death or killing
Group I: control	1	34		0.1	
	2	66		0.2	
	3	270		0.3	
	4	306		0.4	
	5	360		0.4	
	6	38		0.1	
	7	63		0.2	
	8	170		0.2	
	9	508		0.5	
	10	528		0.5	
	11	30		0.1	
	12	126		0.3	
	13	130	Blood chemistry test only		
	14	140			
Group II: single ligation of the choledochus	15	3	.....	.....	Killed by dam one day after operation.
	16	3	.....	.....	
	17	4	.....	.....	
	18	4	.....	.....	
	19	4	.....	.....	
	20	120	f (recanalized)	0.5	Killed 2 months after ope.
	21	120	f (")	1.5	" 5 "
	22	120	f (")	1.9	" 8 "
	23	220	f (")	3.1	" 11 "
Group III-A:	24	35	.....	.....	Died under anaesthsia
Shor-term observation after double ligation and dissection of the choledochus (aged 3-6 weeks and weighing 97 g or less)	25	36	f	3.9	Killed 30 days after ope.
	26	38	f	2.0	" 38 "
	27	35	f	4.0	" 44 "
	28	37	f	3.0	" 52 "
	29	32	f	5.0	" 58 "
	30	48	.....	.....	Died 7 "
	31	47	.....	.....	Died immediately after ope.
	32	53	f	6.0	Killed 35 days after ope.
	33	52	f	2.0	" 43 "
	34	53	f	1.5	" 49 "
	35	54	f	1.5	" 57 "
	36	56	f	9.0	" 60 "
	37	58	c	11.0	" 60 "
	38	45	f	4.0	" 70 "
	39	45	c	13.5	" 78 "
	40	47	c	20.0	" 94 "
	41	39	c	11.0	" 99 "
	42	65	c	10.0	" 110 "
	43	72	f	1.5	" 36 "
	44	70	f	4.0	" 42 "
	45	68	f	2.0	Killed 50 days after ope.
	46	65	f	5.0	" 56 "
	47	62	f	8.0	" 63 "
	48	75	c	20.0	" 63 "
	49	78	c	32.0	" 74 "
	50	66	c	15.0	" 92 "
	51	79	f	2.5	" 27 "
	52	83	f	4.0	" 35 "
	53	88	f	4.0	" 41 "
	54	89	f	4.0	" 55 "
	55	90	.....	.....	Died immediately after ope.
	56	92	f	2.0	Killed 50 days after ope.
	57	94	f	5.0	" 63 "
	58	95	.....	.....	Died immediately after ope.
	59	97	c	10.0	Killed 85 days after ope.
	60	83	c	15.0	" 92 "
	61	80	c	20.0	" 105 "

Table 1.

table by the four legs. The skin of the animals was disinfected with 0.5% hibiten alcohol. The upper and lower abdomen were opened along the midline to fully expose the intraperitoneal cavity.

Table 1. The experimental vesults in rats

Group	Rat No.	Body weight (g)	Shape of extrahepatic bile duct c: cystic type f: fusiform type	Diameter of the extrahepatic bile duct	Date of death or killing
Group Ⅲ-B:	62	160	.....	.....	Died immediately after ope.
Short-term observation after double ligation and dissection of the choledochus (aged 7-11 weeks and weighing 127-273 g)	63	169	f	5.5	Killed 20 days after ope.
	64	184	f	6.0	" 26 "
	65	170	f	6.0	" 34 "
	66	130	f	9.0	" 47 "
	67	135	c	12.0	" 55 "
	68	127	f	4.0	" 16 "
	69	129	f	5.0	" 22 "
	70	130	f	4.0	" 30 "
	71	135	f	6.5	" 33 "
	72	137	f	7.5	" 37 "
	73	140	f	4.0	" 45 "
	74	192	f	3.0	" 39 "
	75	195	f	3.0	" 47 "
	76	196	f	6.0	" 50 "
	77	196	f	7.0	" 54 "
	78	198	f	7.5	" 62 "
	79	199	f	9.0	" 89 "
	80	204	f	5.0	" 38 "
	81	209	f	5.0	" 41 "
	82	214	f	6.0	" 45 "
	83	215	f	6.5	" 53 "
	84	216	f	5.0	" 80 "
	85	236	f	7.0	" 51 "
	86	274	f	6.0	" 95 "
	87	272	.....	.....	Died immediately after ope.
Group Ⅲ-C:	88	430	f	4.0	Died 7 days after ope.
Short-term observation after double ligation and dissection of the choledochus (weighing 430-570 g)	89	472	f	5.0	" 13 "
	90	494	f	5.0	" 16 "
	91	560	f	4.0	" 9 "
	92	568	f	5.0	" 21 "
	93	570	.....	.....	Died immediately after ope.
Group Ⅱ:	94	94	scar		Killed 8 months after ope.
Long-term observation after double lization and dissection of the choledochus	95	90	scar		" 12 "
	96	92	scar		" 12 "
	97	68	scar		" 9 "
	98	70	c	9.0	" 7 "
	99	70	c	15.0	" 7 "
	100	71	scar		" 7 "
	101	72	scar		" 7 "
	102	74	scar		" 7 "
	103	75	scar		" 7 "
	104	78	scar		" 7 "
	105	79	scar		" 7 "
	106	92	scar		" 13 "
	107	95	scar		" 14 "
	108	86	scar		" 15 "

Group	Rat No.	Duration from the dissection to the anastomosis	Method of anastomosis	Date of death or killing
Group Ⅰ:	109	2 months	cyst duodenostomy	Died 1 days after operation
anastomosed cyst-small intestine	110	2 "	"	" 2 "
	111	2 "	cyst duodenostomy with jejunal interposition	" 3 "
	112	3.5 "	cyst duodenostomy	" 5 "
	113	5.5 "	cyst jejunostomy	" 7 "
	114	5.5 "	"	Died immediately after operation
	115	3 "	cyst duodenostomy	Killed 3.5 months after operation ○
	116	3 "	"	Died immediately after opration
	117	3 "	"	Killed 3.5 months after operation ×
	118	3 "	"	" 6 " ○
	119	5 "	"	" 4.5 " ×
	120	2.5 "	"	" 5 " ○
	121	2.5 "	"	" 5 " ×
	122	5 "	"	" 6 " ×
	123	5 "	"	" 6 " ○

○ : anastomosed orifice open
× : anastomosed orifice closed

Table 2. Data of the Group I

	n	mean ± S.D.
T.B.	8	0.32± 0.09mg/dℓ
D.B.	8	0.11± 0.02mg/dℓ
ALP	7	55.03± 28.60 IU/L
GOT	7	136.86± 38.77 IU/L
GPT	7	27.71± 9.16 IU/L
T.CHO	4	96.00± 37.44mg/dℓ
AMY	8	1420.12±316.11 IU/L
MAO	3	5.67± 1.15 IU/L
Bile duct (diameter)	12	0.28± 0.15mm

(i) Group I : control

(ii) Group II : At the level of upper margin of the pancreas, the choledochus was ligated with 7-0 Tycron® (Fig. 1).

(iii) Group III: At the level of upper margin of the pancreas, the choledochus was ligated with Tycron® and dissected with a scalpel. (Fig. 2)
The animals were observed for 16-110 days after operation.

(iv) Group IV: At the level of upper margin of the pancreas, the choleclochus was ligated and dissected in the same way as (iii). The animals were observed for 7-15 months after operation.

(v) Group V : Rats with successful cystic dilatation of the extrahepatic bile duct as in (iii) were subjected to cyst-duodenostomy by continuous suture with 7-0 Tycron® (Fig. 3).

The abdominal wall in all the operated animals (ii)~(v) was closed by interrupted suturing with 3-0 silk thread, and disinfected of the skin with 0.5% hibiten alcohol. Antibiotics were not given.

3. Postoperative management

Young rats aged 3-6 weeks and weighing 97 g or less were kept warm on an electric carpet during and after operation. They were housed in cages after careful observation for restoration of movement. Rats aged 7 weeks or more and weighing 100 g or more were housed in cages immediately after operation.

4. Test items

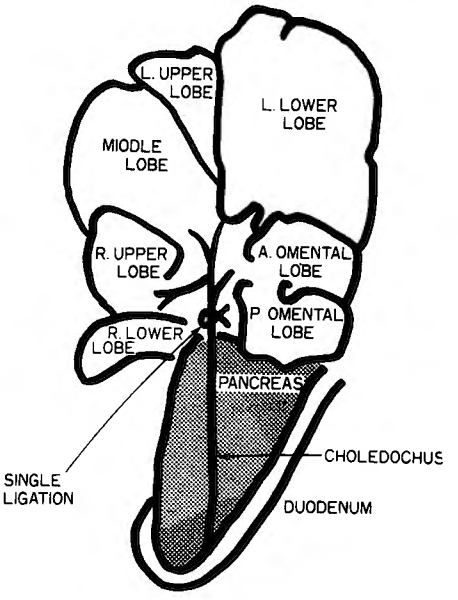

Fig. 1. Group II: Single ligation of the choledochus with 7-0 Tycron®

a. Macroscopic observation of the extrahepatic bile duct and the liver was done under laparotomy.
b. Histological study

After completion of the macroscopic observation, the liver and extrahepatic bile duct were removed with a surgical knife, preserved in 10% buffered formalin, embedded in paraffin, sectioned

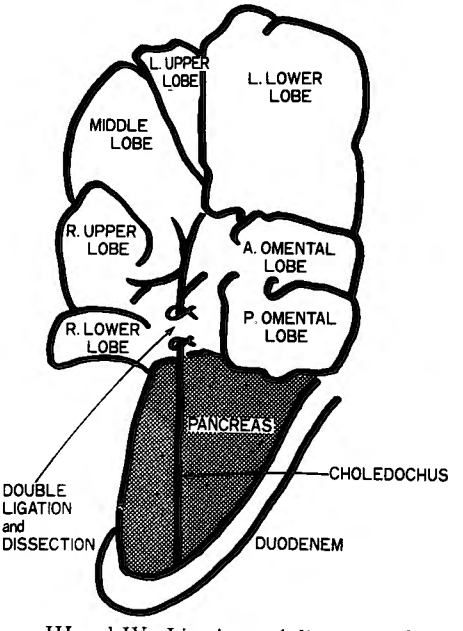

Fig. 2. Groups III and IV: Ligation and dissection of the choledochus

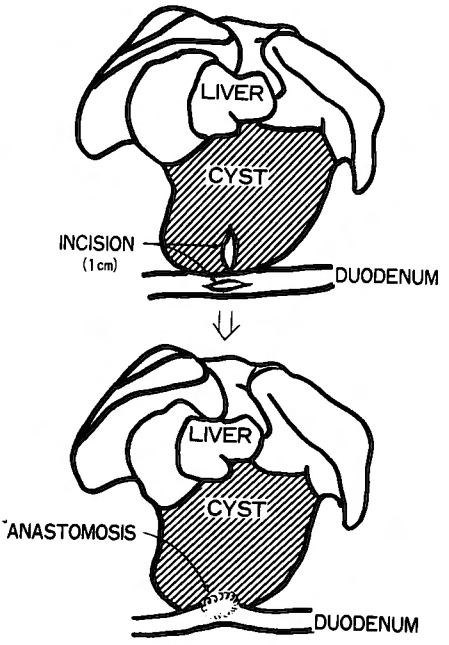

Fig. 3. Group V: Continuous suture of the cyst-duodenum

at 8μ, stained with haematoxylin-eosin, elastica-v. Gieson and azan and examined microscopically.

For the liver, HE-stained specimens were magnified 100 times to count the number of bile ductuli per portal area (i. e. B/P ratio).

c. Biochemistry tests

Under ether anesthesia, the blood was collected from the heart to perform the following tests :

(1) Total bilirubin values were determined by the diazoalcohol method with a Hitachi 191 spectrophotometer.

(2) Alkaline phosphatase values were determined by the Kind-King method with a Hitachi 716 autoanalyzer.

(3) GOT values were determined by the U.V. method with a Hitachi 716 autoanalyzer.

(4) GPT values were determined by the U.V. method with a Hitachi 716 autoanalyzer.

(5) Total cholesterol values were determined with a Hitachi 716 autonanalyzer.

(6) Serum amylase values were determined by the iodometric method with a Hitachi 191 spectrophotometer.

(7) MAO values were determined with a Hitachi 701 spectrophotometer.

Results

1. **Group II** (single ligation of the choledochus) (Table 3)

Five out of the 9 rats were housed with their dam after operation because they were too nursing. The 5 young were eaten by their dam within one day. The remaining 4 weighing

Table 3. Data of the Group II

	n	mean±SD
T.B.	4	0.26± 0.05 mg/dℓ
D.B.	4	0.08± 0.03 mg/dℓ
ALP	4	25.50± 3.21 IU/L
GOT	4	100.0± 40.2 IU/L
GPT	4	23.25± 10.05 IU/L
T.CHO	4	132.25± 29.01 mg/dℓ
AMY	4	1701.50±141.16 IU/L
MAO	3	8.67± 8.96 IU/L
Bile duct (diameter)	4	1.75± 1.07 mm

120–220 g were observed for 2, 5, 8 and 11 months respectively after operation.

a) Extrahepatic bile duct: slight fusiform type biliary dilatation was observed from the ligated part to proximal choledochus. The choledochus was recanalized in all the rats.

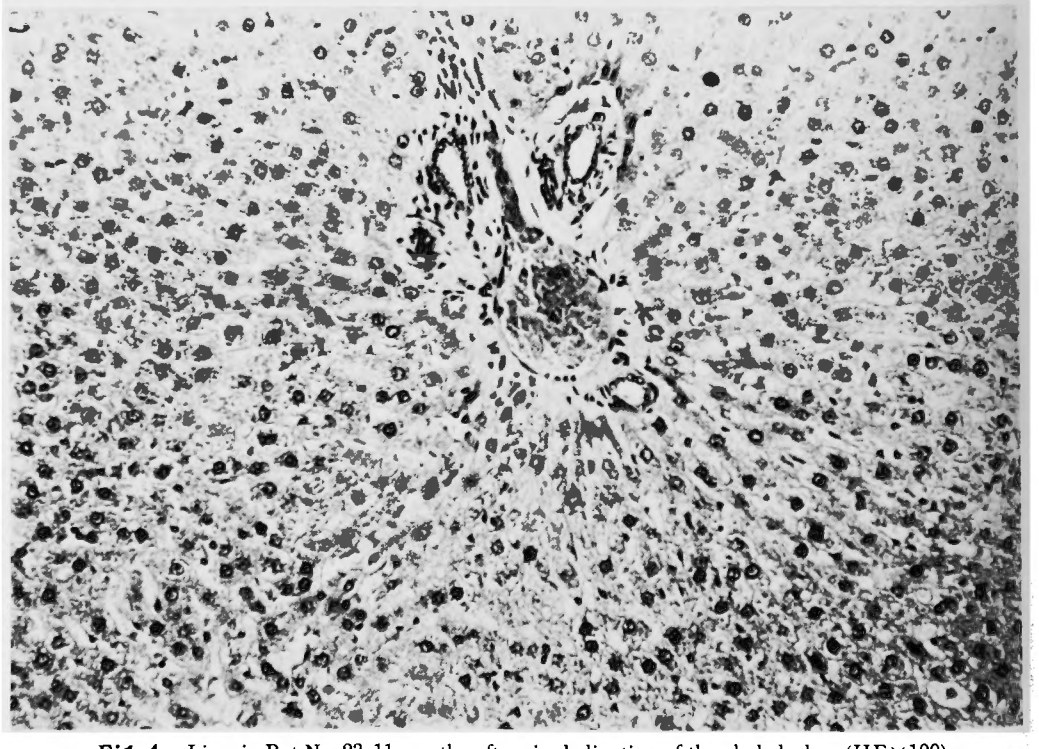

Fig. 4. Liver in Rat No. 23, 11 months after single ligation of the choledochus (HE×100)

b) Histological findings

(1) Liver (Fig, 4):

No significant differences were noted between Group I and Group II.

(2) Extrahepatic bile dnct (Fig. 5):

The epithelium of the bile duct was intact. No degeneration or destruction of the elastic fibers were seen in any of the rats.

c) Biochemistry test.

(1) Total bilirubin values were 0.26±0.05 mg/dl (mean±S. D.). No significant differences were observed between Group I and Group II.

(2) Alkaline phosphatase

Alkaline phosphatase valuse were 25.50±3.21 IU/L (mean±S.D.) No significant differences were ovserved berween Group I and Group II.

(3) GOT values were 100.0±40.2 IU/L (mean±S. D.). No significant differences were observed between Group I and Group II.

(4) GPT values were 23.25±10.05 IU/L (mean±S. D.). No significant differences were observed between Group I and Group II.

(5) Total cholesterol values were 132.25±29.01 mg/dl (mean±S. D.). No significant differences were observed between Group I and Group II.

(6) Serum amylase values were 1701.50±141.16 IU/L (mean±S. D.). These values were

Fig. 5. Extrahepatic bile duct of Rat No. 23, 11 months after single ligation of the choledochus (HE×100)

Table 4. Data of the Group III-A

	n	mean±SD
T.B.	28	4.02± 3.05 mg/dℓ
D.B.	28	3.15± 2.50 mg/dℓ
ALP	24	56.91± 13.12 IU/L
GOT	23	268.22±160.71 IU/L
GPT	23	43.52± 35.02 IU/L
T.CHO	20	69.05± 23.69 mg/dℓ
AMY	26	1825.38±406.54 IU/L
MAO	20	10.95± 6.36 IU/L
Bile duct (diameter)	33	7.92± 7.05 mm

significantly higher in Group II than in Group I.

Histological observations showed that cystic dilatation did not occur in Group II. No abnormal changes were noted in total bilirubin, alkaline phosphatase, GOT, GPT or total cholesterol, but significant increase in serum amylase was observed.

2. **Group III** (short-term observation afrter ligation and dissection of the choledochus) (Tables 4, 5)

Table 5. Data of the Group III-B

	n	mean±S.D.
T.B.	24	3.13± 3.07 mg/dℓ
D.B.	24	2.44± 2.59 mg/dℓ
ALP	19	61.52± 14.60 IU/L
GOT	19	270.00±144.44 IU/L
GPT	19	59.32± 24.15 IU/L
T.CHO	19	86.74± 29.76 mg/dℓ
AMY	22	2032.27±332.34 IU/L
MAO	12	15.91± 8.47 IU/L
Bile duct (diameter)	24	6.06± 2.03 mm

Animals of Group III were observed for 16 to 110 days after ligation and dissection. The structure of the extrahepatic bile duct differed according to age and weight at the time of operation as described later.

Group III consisted of folloing 3 subgroups :

Group III-A : Animals aged 3-6 weeks and weighing 32-97 g at the time of operation were used.

Group III-B : Animals aged 7-11 weeks and weighing 127-274 g at the time of operation were used.

Group III-C · Animals weighing 430-570 g at the time of operation were used.

a) Structure of the extrahepatic bile duct (Fig. 6)

(1) Group III-A

Fusiform type dilatation was seen from 27 days after operation (Fig. 7) and cystic dilatation was seen from 60 days after operation.

Cystic dilatation (32 mm in diameter) 100 times that in the contol was found in Rat No. 49. (Fig. 8).

(2) Group III-B

Fusiform type dilatation was seen slightly from 16 days after operation. (Fig. 9)

(3) Group III-C

Fusiform type dilatation was seen in all animals and all died within 1-3 weeks after ligation

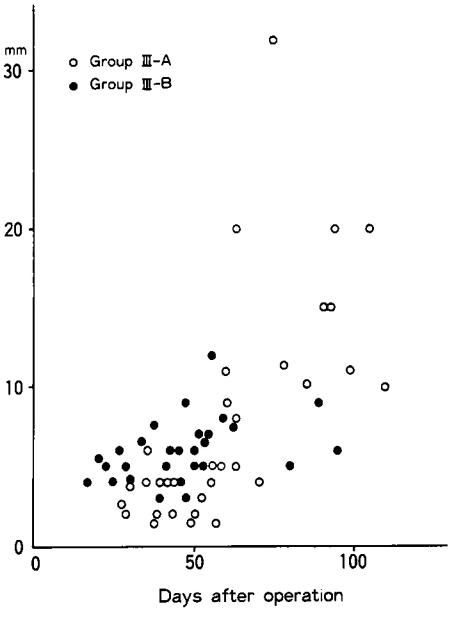

Fig. 6. Diameter of the extrahepatic bile duct

○ Group III-A (short-term observation after ligation and dissection of the choledochus: weighing 32-97g)

● group III-B (short-term observation after ligation and dissection of the choledochus: weighing 127-273g)

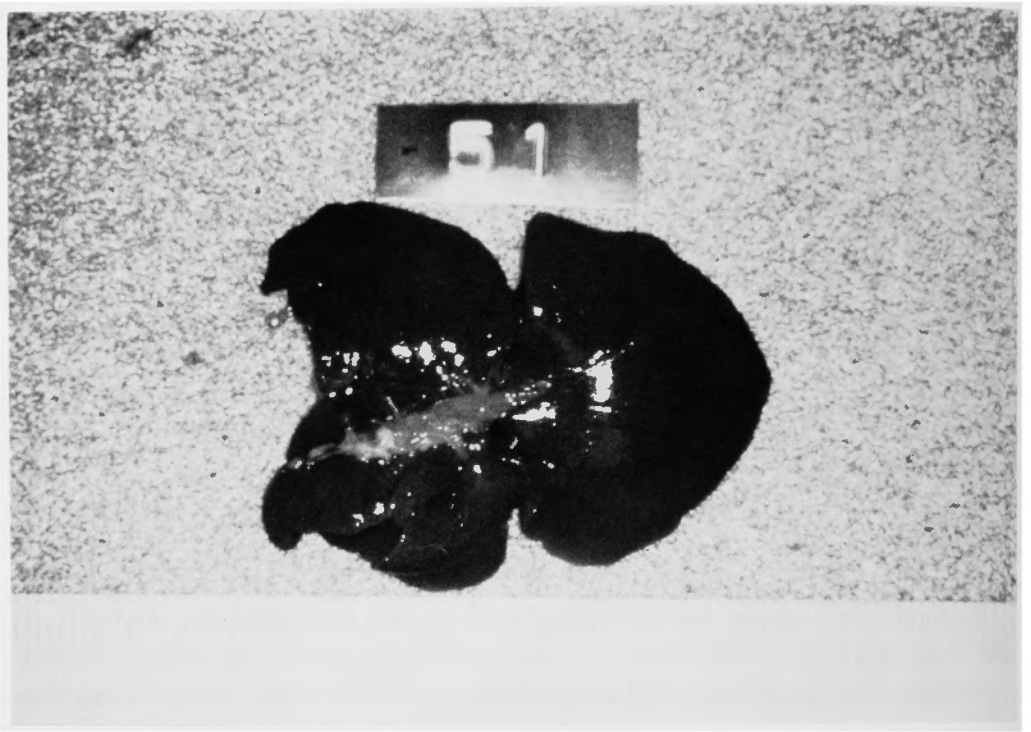

Fig. 7. Fusiform type dilatation in Rat No. 51, 27 days after ligation and dissection of the choledochus

and dissection of the choledochus.

b) Histological findings

(1) Liver

No significant differences were noted between Groups III-A and -B. The following findings were common to Groups III-A and -B.

○No dilatation of the intrahepatic bile duct

○Marked proliferation of the bile cancaliculi (Fig. 10).

B/P ratio increased linearly immediately after dissection, reached 40 two months after dissection, and increased slightly thereafter (Fig, 11).

○Gradual fibrosis after dissection

○Hepatic cirrhosis picture uncertain

(2) Extrahepatic bile duct

Loss of the epithelium of the bile duct, thickening and degeneration of the bile duct wall, especially destruction of the elastic fibers were observed (Fig. 12). These changes were more remarkable in rats with cystic dilatation than in those with fusiform type dilatation (Fig. 13). (Histological examination could not be done in Group III-C because all animals died.)

c) Biochemistry tests

(1) Total bilirubin values were 0.32 ± 0.09 mg/dl in Group I; 4.02 ± 3.05 mg/dl; in group III

Fig. 8. Cystic dilatation in Rat No. 49, 74 days after ligation and dissection of the choledochus

A ; 3.13±3.07 mg/dl in Group III-B. These values were significantly higher than those in group I. However, no signigicant differences were noted between Groups III-A and -B (Fig. 14 and 15).

(2) Alkaline phosphatase

Alkaline phosphatese values were 56.91±13.12 IU/L in Group III-A ; 61.52±14.60 IU/L in Group III-B ; and 55.03±28.60 IU/L in Group I.

No significant differences were noted between Groups III-A and -B and Group I or between Groups III-A and -B.

(3) GOT values were 268.22±160.71 IU/L in Group III-A; 270.00±144.44 IU/L in Group III-B ; and 136.86±38.77 IU/L in Group I. These values were significantly higher in Groups III-A and -B than in Group I. However, no significant differences were noted between Groups III-A and -B.

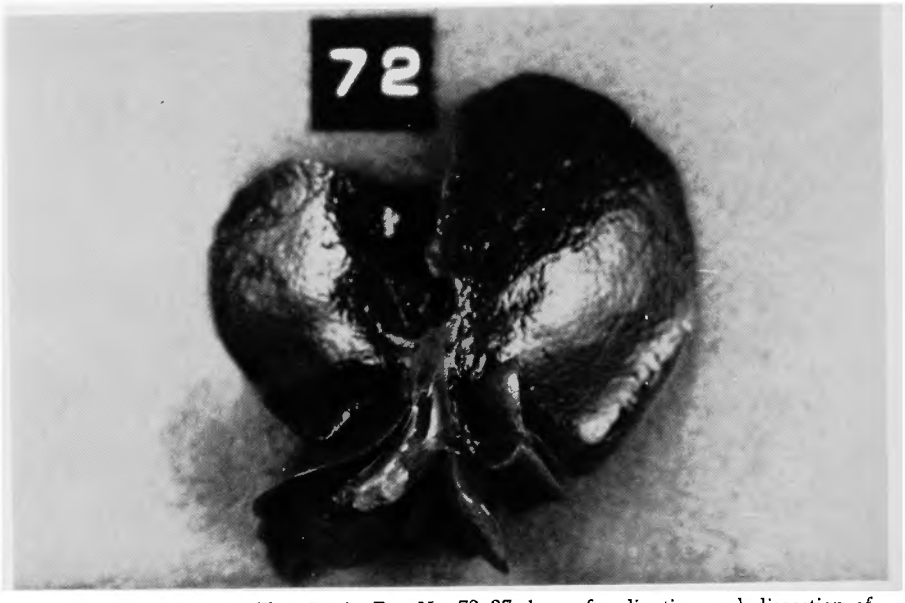

Fig. 9. Fusiform type dilatation in Rat No. 72, 37 days after ligation and disscetion of the choledochus

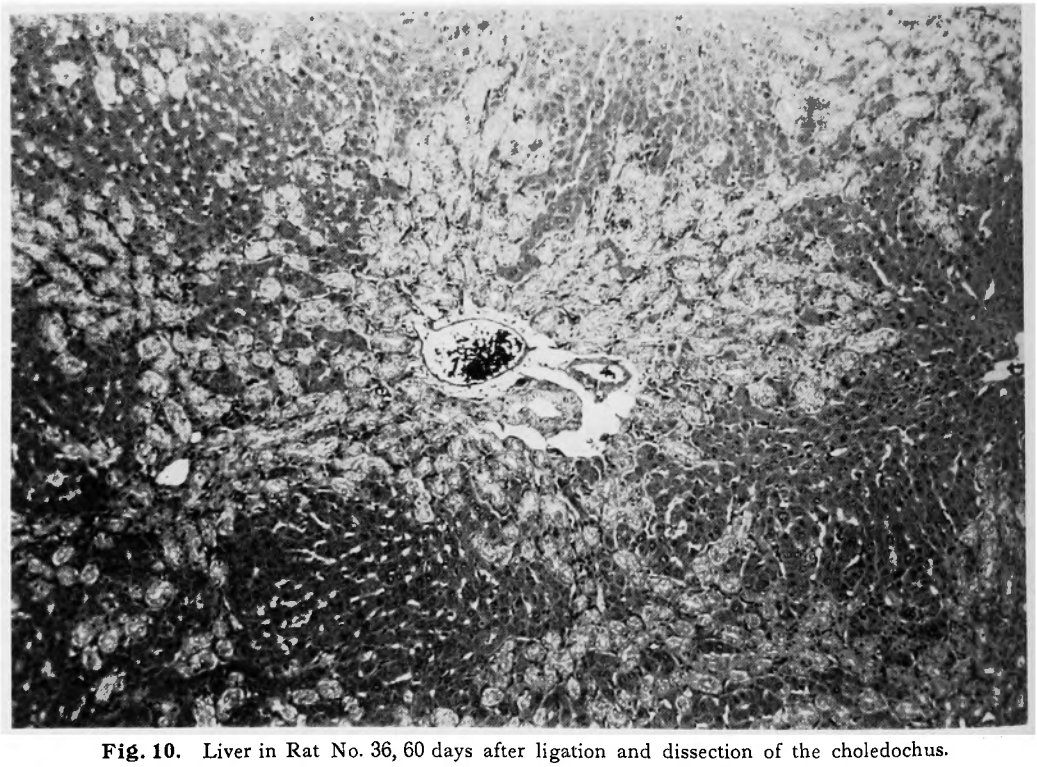

Fig. 10. Liver in Rat No. 36, 60 days after ligation and dissection of the choledochus. —Marked proliferation of bile ductuli is evident (HE×40)

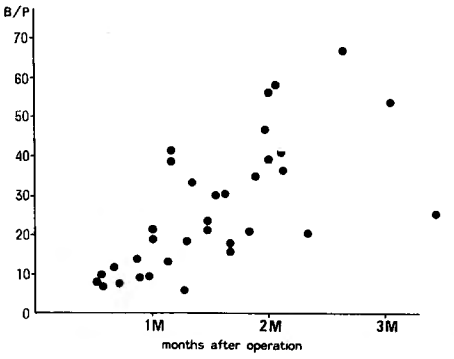

Fig. 11. A count of bile ductuli/portal area (B/P ratio) in Groups III-A and -B

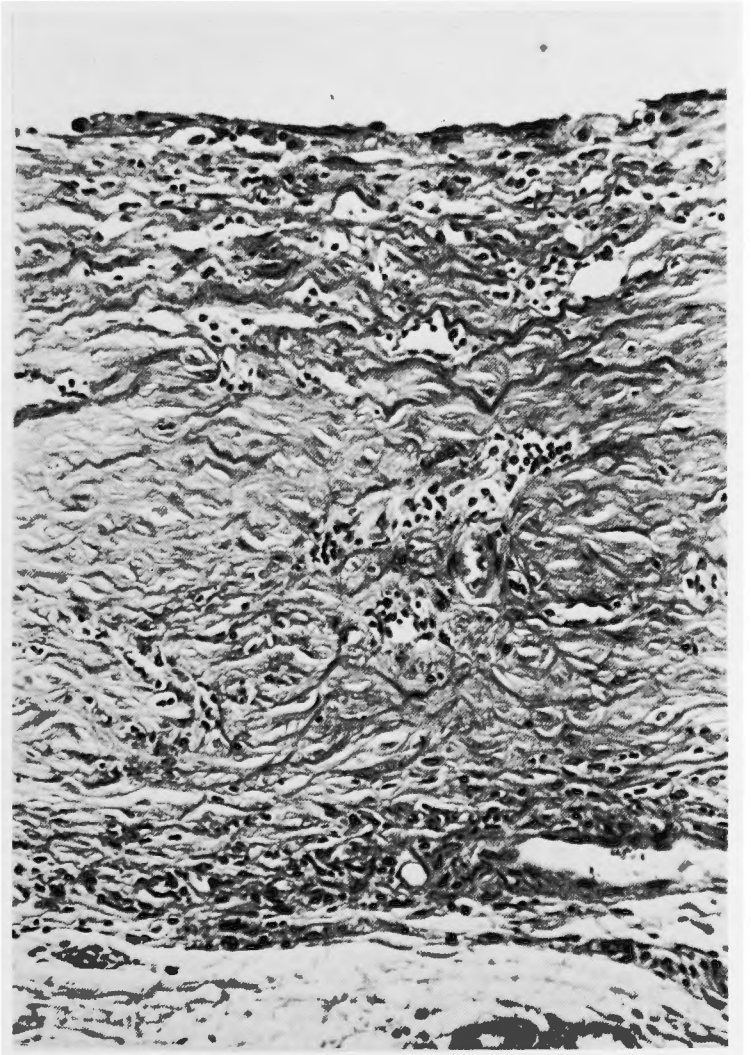

Fig. 12. Dilated bile duct wall in Rat No. 40, 94 days after ligation and dissection of the choledochus (elastica-V. Gieson×100) showing degeneration and thichening of the bile duct wall. The epithelium is engulfed.

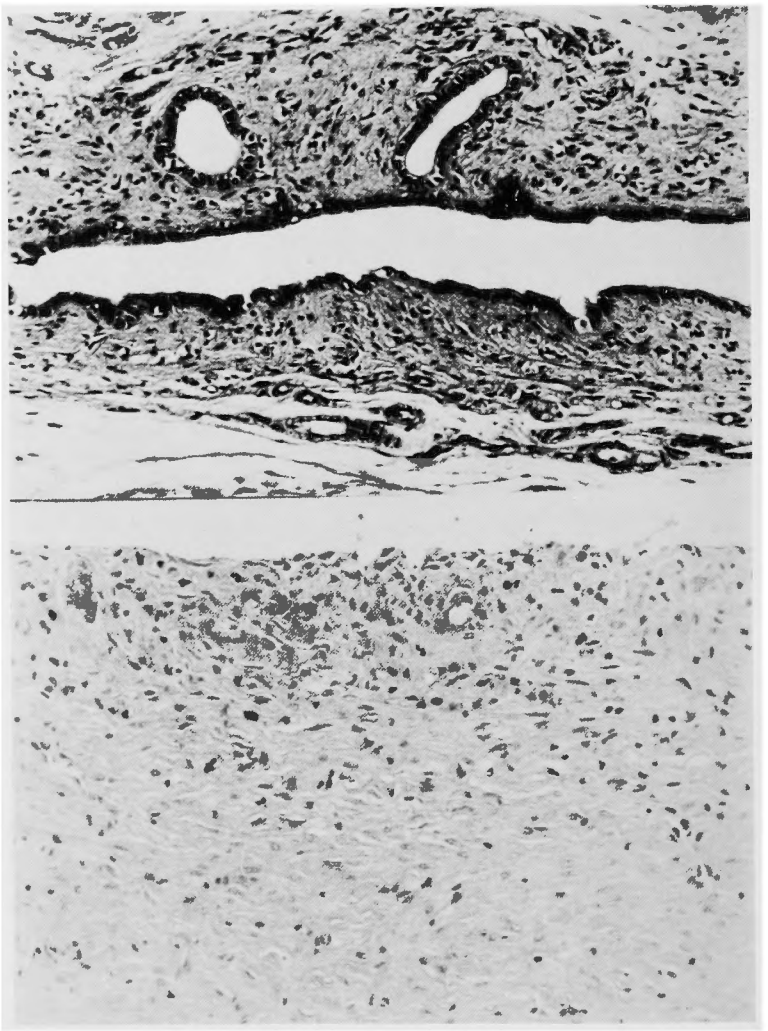

Fig. 13. Upper-Fusiform type dilatation of the bile duct wall in Rat No. 33 after ligation and dissection of the choledochus —The epithelium is intact. (HE×40)
Lower-Cystic dilatation of the bile duct wall in Rat No. 37 after ligation and dissection of the choledochus —The epithelium is completely lost. (HE×40)

(4) GPT values were 43.52±35.02 IU/L in Group III-A ; 59.32±24.15 IU/L in Group III-B and 27.71±9.16 IU/L in Group I. No significant differences were noted between Group III-A and Group I. These values were significantly higher in Group III-B than in Group I. No significant differences were noted between Groups III-A and -B.

(5) Total cholesterol walues were 69.05±23.69 mg/dl in Group III-A ; 86.74±29.76 in Group III-B ; and 96.00±37.44 mg/dl in Group I. No significant differences were noted between Groups III-A and -B and Group I. These values were significantly higher in Group III-B than in Group III-A.

(6) Serum amylase values were 1825.38±406.54 IU/L in Group III-A ; 2032.27±332.34 IU/L

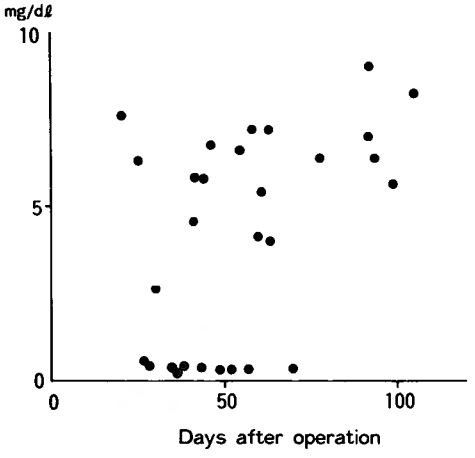

Fig. 14. Total bilirubin
Group III-A (short-term observation after ligation and dissection of the choledochus)
(Rats aged 3-6 weeks and weighing 32-97 g)

in Group III-B ; and 1420.12±316.11 IU/L in Group I. These values were significantly higher in Groups III-A and -B than in Group I.

However, no significant differences were noted between Groups III-A and -B.

(7) MAO values were 10.95±6.30 IU/L in Group III-A ; 15.91±8.47 IU/L in Group III-B ; and 5.67±1.15 IU/L in Group I. These values were significantly higher in Groups III-A and -B than in Group I.

However, no significant differences were noted between Groups III-A and -B. (Biochemistry tests could not be done in Group III-C because all animals died.)

3. **Group IV** (long-term observation after ligation and dissection of the choledochus) (Table 6)

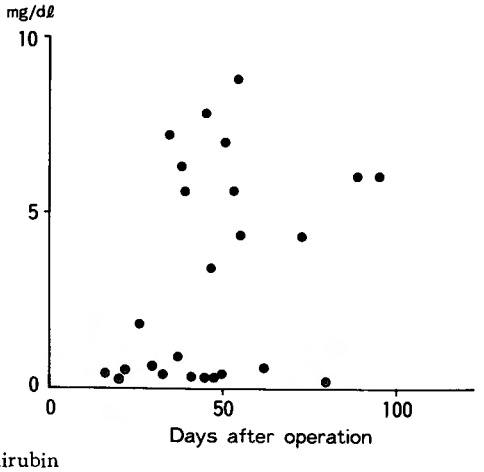

Fig. 15. Total bilirubin
Group III-B (short-term observation double after ligation and dissection of the choledochus)
(Rats aged 7 weeks or more and weighing 127-274g)

Table 6. Data of the Group IV

	n	mean ± S.D.
T.B.	15	9.00± 3.05 mg/dℓ
D.B.	15	7.28± 2.65 mg/dℓ
ALP	15	42.27± 9.74 IU/L
GOT	15	442.93±157.41 IU/L
GPT	15	60.47± 20.87 IU/L
T.CHO	15	153.60 ± 39.87 mg/dℓ
AMY	15	1127.93±281.66 IU/L
MAO	14	15.00± 6.78 IU/L

Fifteen rats aged 6 weeks or less and weighing less than 100 g with ligated and dissected choledochus were observed for 7-15 months.

a) Extrahepatic bile duct:

Cystic dilatation (diameter: 15 mm and 9 mm) was seen in 2 (observed for 7 months after dissection) of the 15 animals. Cyst was scarred in the remaining 13.

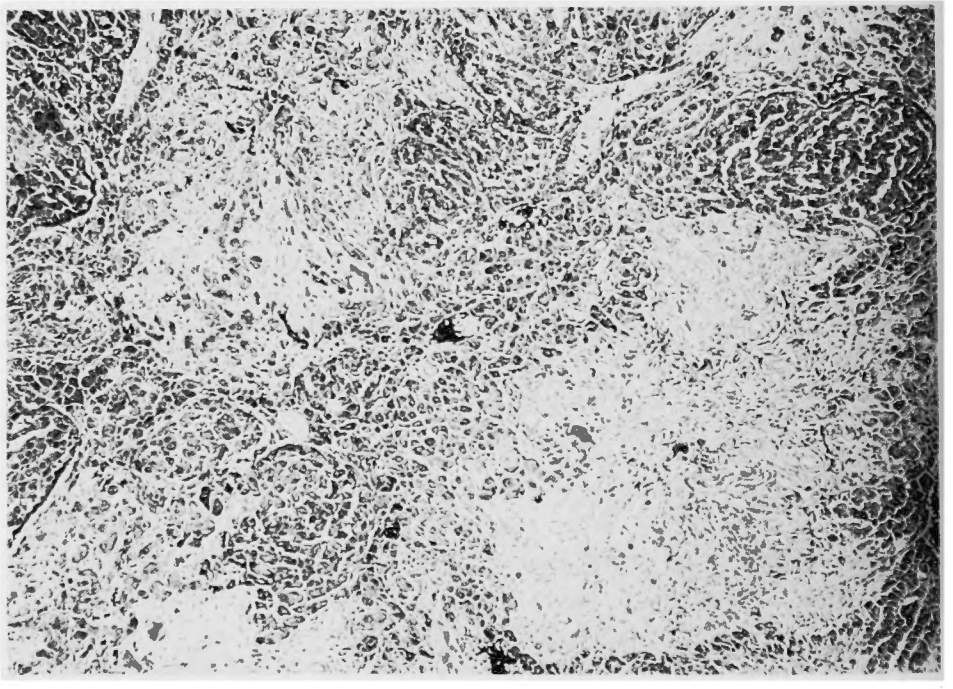

Fig. 16. Liver in Rat No. 97, 9 months after ligation and dissection of the choledochus—The architecture of the hepatic cells is in disarry with formation of pseudolobules, and marked fifrosis (HE×40)

b) Histological findings

(1) Liver : Hepatic cell architecture in disarray, pseudolobules, fibrosis and vacuolization were observed. These changes resembled those in patients with hepatic cirrhosis (Fig. 16).

(2) Extrahepatic bile duct :

The epithelium of the bile duct was lost and the wall was degenerated in 2 animals with cyst. No cyst remained in the other 13, but cyst were scarred in the porta hepatis (Fig. 17). No carcinoma was seen in any of the animals.

c) Biochemistry tests

(1) Total bilirubin values (Fig. 18) were 9.00 ± 3.05 mg/dl, significantly higher than in Group I, and Groups III-A and -B.

(1)' Direct bilirubin values were 7.28 ± 2.65 mg/dl, significantly higher than those in the control and Groups III-A and -B.

(2) Alkaline phosphatase values were 42.27 ± 9.74 IU/L. No significant differences were noted between Group IV and Groups III-A and -B.

(3) GOT values were 442.93 ± 157.41 U/L, signigicantly higher than those in Group I, and Groups III-A and -B.

(4) GPT values were 60.47 ± 20.87 IU/L, significantly higher than those in Group I. No significant differences were observed between Group IV and Group III-A and -B.

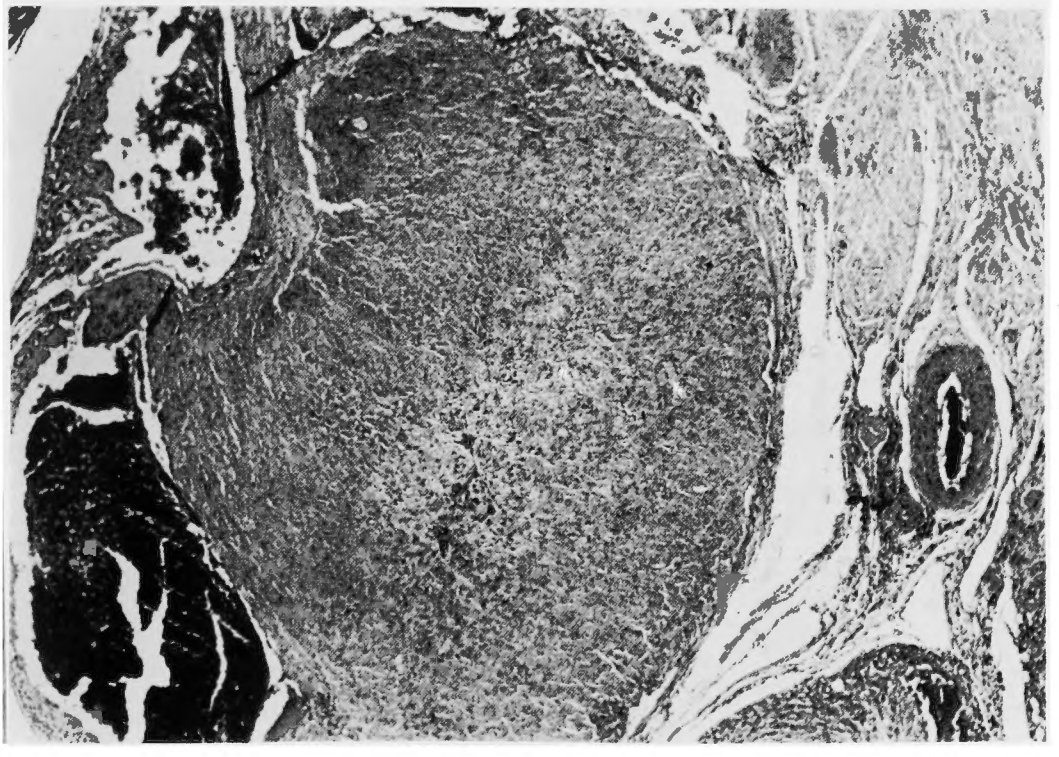

Fig. 17. Porta hepatis of Rat No. 108, 15 months after ligation and dissection of the choledochus —Cyst scar remain (HE×40)

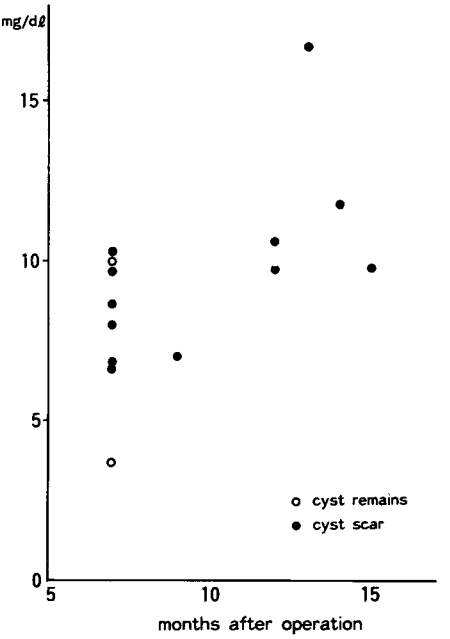

Fig. 18. Total bilirubin
Group IV (long-term observation after and dissection of the choledochus)
● cyst scar ○ cyst remains

(5) Total choledsterol values were 153.60±39.87 mg/dl, significantly higher than those in Group I, and Groups III-A and -B.

(6) Serum amylase values were 1127.93±281.96 IU/L, significantly lower than those in Group I and Groups III-A and -B.

(7) MAO values were 15.00±6.78 IU/L, significantly higher than those in Groups I. No significant differences were noted between Group IV and Groups III-A and -B.

4. **Group V** (cyst-duodenostomy)

Anastomosis (cyst-small intestine) was performed in 15 rats. The abdomen was opened 2-5.5 months after ligation and dissection of the choledochus and choledochal cyst was confirmed ; 12 had cyst-duodenostomy ; 2, cystjejunostomy (Roux-Y anastomosis) ; and 1, cyst-duodenostomy with jejunal interposition. Of these rats, 4 with cyst-duodenostomy, 2 with cyst-jejunostomy and 1 with cyst-cuodenostomy with jejunal interposition died within one week after operation. The 8 rats with cyst-duodenostomy which survived 3.5 months or longer were observed for 3.5-6 months after operation for the following studies :

a) Macroscopical findings of extrahepatic bile duct and anastomosis

The anastomotic orifice was open in 3 of these 8 rats : The orifice of the anastomosis was 5 mm in diameter in Rat No. 113 (3.5 months after operation), 9 mm in Rat No. 118 (6 months after operation), and 6 mm in Rat No. 120 (5 months after operation). The orifice was closed in the remaining 5. However, the bile was present in the duodenum at the time of sacrifice in Rat No. 123 (6 months after operation). And this data similar to those with open orifice were obtained

in the biochemistry tests as mentioned later. Therefore, this rat was divided into Group V-B (open orifice, Nos. 115, 118, 120 and 123) and Group V-A (closed orifice, Nos. 117, 119, 121 adn 122) and subjected to following tests.

b) Histological findings

(1) Liver:

In Group V-A (Fig. 19), the liver cells were vacuolated and hepatic architecture was in disarray. These findings were very similar to those in Group IV. In Group V-B (Fig. 20), slight fibrosis was noted, centering on the portal tract but the parencymal cells were not changed to any extent.

(2) Extrahepatic bile duct:

In Group V-A, scar were noted in the cyst (Fig. 21). In Group V-B, cyst was constricted from the time of anastomosis and the wall was thickened. Carcinoma was not observed in Groups V-A or V-B.

c) Biochemistry tests (Tables 7, 8)

(1) Total bilirubin values were 8.97 ± 1.56 mg/dl in Group V-A and were significantly higher than those in Group I, Group II, Group III-A, Group III-B, and Group V-B. No significant differences were noted between Group IV and Group V-A. Total bilirubin values were 0.31 ± 0.03 mg/dl in Group V-B and were significantly lower than those in Group III-A, Group III-B,

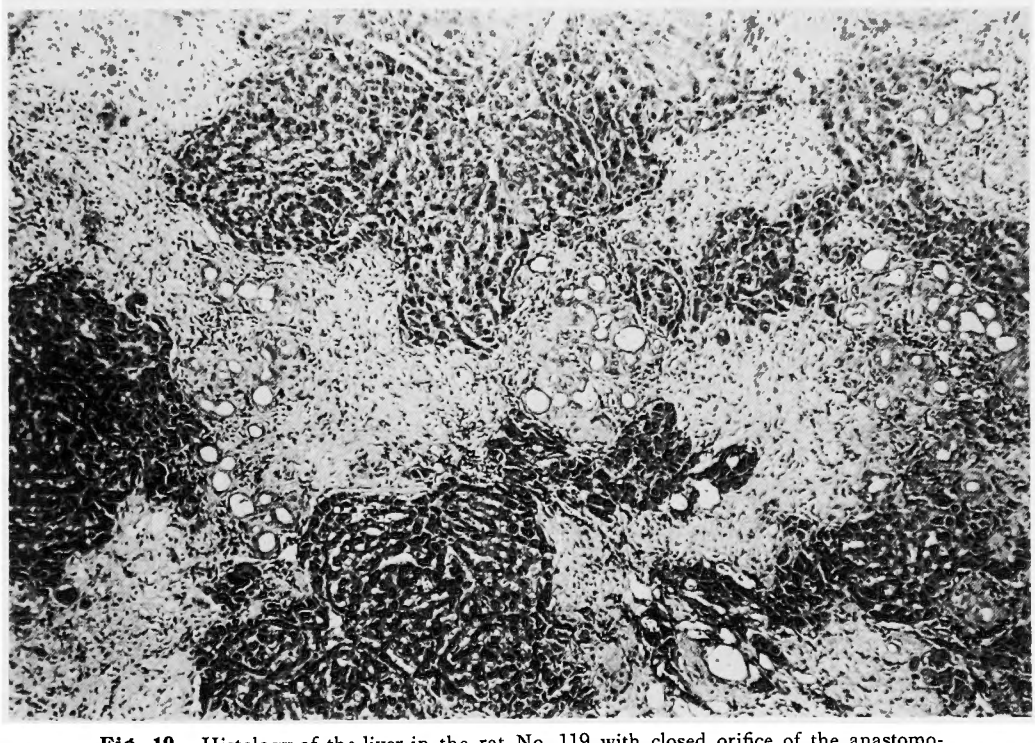

Fig. 19. Histology of the liver in the rat No. 119 with closed orifice of the anastomosis, 3 months after anastomosis. —Architecture of the hepatic cells is in disarray with marked fibrosis and pseudolobules. (HE×40)

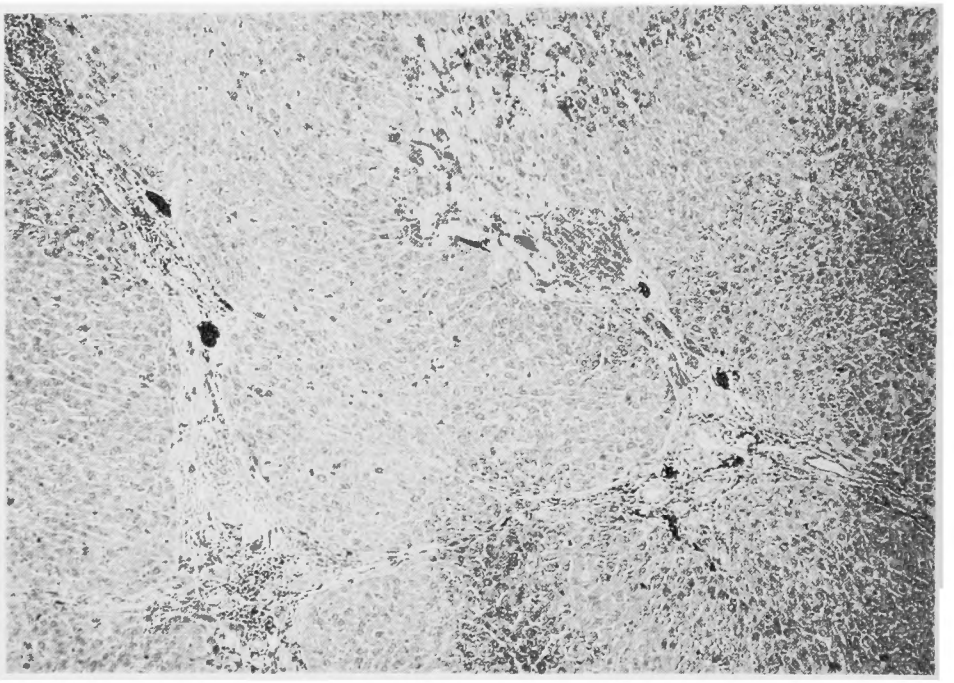

Fig. 20. Histology of the liver in Rat No. 120 with open orifice of the anastomosis, 3 months after the anastomosis —Slight fibrosis and cell infiltration in the portal area and proferated bile ductuli; hepatic cells unchanged. (HE×40)

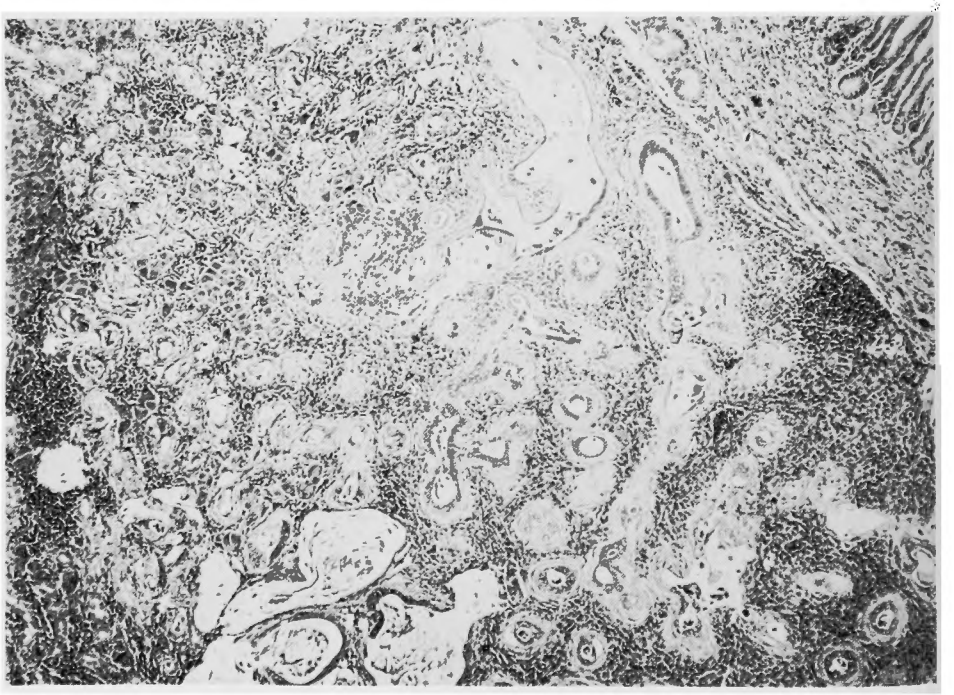

Fig. 21. Histology of the part of cyst-duodenostomy of Rat No. 117, 3.5 months after anastomosis. —Marked cell infiltration and cyst scars remain (HE×40)

Table 7. Data of the Group V-A

	n	mean±S.D.
T.B.	4	8.97± 1.56 mg/dℓ
D.B.	4	6.95± 1.48 mg/dℓ
ALP	4	42.73± 8.27 IU/L
GOT	4	395.75± 65.98 IU/L
GPT	4	50.25± 16.52 IU/L
T.CHO	4	169.25± 15.00 mg/dℓ
AMY	4	1295.00±108.40 IU/L
MAO	4	16.50± 8.58 IU/L

Group IV. No significant differences were noted between Group V-B and Group I.

(1) Direct bilirubin values were 6.95±1.48 mg/dl in Group V-A and were significantly higher than those in Group I, Group II, Groups III-A and -B, and Group V-B.

No significant differences were noted between Group IV and Group V-A. Direct bilirubin values were 0.10±0.0 mg/dl in Group V-B and were significantly lower than those in Groups III-A and -B, and Group IV.

No significant differences were noted between Group V-B and Group I or Group II.

(2) Alkaline phosphatase values were 42.73±8.72 IU/L in Group V-A and were significantly higer than those in Group II, and were signiciantly lower than those in Groups III-A and -B. No significant differences were noted between Group V-A and Group IV or Group V-B.

Table 8. Data of the Group V-B

	n	mean±S.D.
T.B.	4	0.31± 0.03 mg/dℓ
D.B.	4	0.10± 0.0 mg/dℓ
ALP	4	46.28± 2.37 IU/L
GOT	4	145.75± 35.68 IU/L
GPT	4	31.00± 17.19 IU/L
T.CHO	4	145.25± 66.42 mg/dℓ
AMY	4	1405.00±208.25 IU/L
MAO	4	23.50± 5.80 IU/L

Alkaline phosphatase values were 46.28±2.37 IU/L in Group V-B and were significantly higher than those in Group II and were significantly lower then those in Groups III-A and-B. No significant differences were noted between Group V-B and Group I or Group IV.

(3) GOT values were 395.75±65.98 IU/L in Group V-A and were significantly higher than those in Group I, Group II, Groups III-A and -B, Group V-B. No significant differences were noted between Group V-A and Group IV.

GOT values were 145.75±35.68 IU/L in Group V-B and were signiciantly lower than Groups III-A and -B and Group IV. No significant differences were noted between Group V-B and Group I or Group II.

(4) GPT values were 50.25±16.52 IU/L in Group V-A and were significantly higher than those in Group II. No significant differences were noted between Group V-A and Group I, Groups III-A, and -B, Group IV or Group V-B. GPT values were 31.00±17.19 IU/L in Group V-B, and were significantly lower than those in Group III-B and Group IV. No significant differences were noted between Group V-B and Group, Group II, or Group III-A.

(5) Total cholesterol:

Total cholesterol values were 169.25±15.00 mg/dl in Group V-A and were significantly higher than those in Group I and Group III-A and -B.

No significant differences were noted between Group V-A and Group II, Group IV and Group V-B. Total cholesterol values were 145.25±66.42 mg/dl in Group V-B. No significant differences were noted between Group V-B and Group I, Group II, Groups III-A and -B or Group IV.

(6) Serum amylase values were 1295.00±108.40 IU/L in Group V-A and were significantly lower than those in Group II and Groups III-A and -B. No significant differences were noted between Group V-A and Group I, Group IV or Group V-B. Serum amylase values were 1405.00±208.25 IU/L in Group V-B and were significantly lower than those in Groups III-A and -B. No significant differences were noted between Group V-B and Group I, Group II or Group IV.

(7) MAO values were 16.50±8.58 IU/L in Group V-A. No significant differences were noted between Group V-A and Group I, Group II, Group IV and Group V-B. MAO values were 23.50±5.80 IU/L in Group V-B and were significantly higher than those in Group I and Group III-A. No significant differences were noted between Group V-B and Group II, Group III-B or Group IV.

Discussion

Since the first description of congenital biliary dilatation by Vater[24] in 1723, many theories concerning the etiology of the disease have been proposed, Shallow[20] and Alonso-Lej[1] collected these data and discussed the etiology of the cystic type of the disease.

Yotsuyanagi's theory[25] (based on inequality of epithelial proliferation at the stage physiological epithelial occulusion) has often been cited by many authors. Landing[14] stated that the disease is caused by factors not unlike those in neonatal hepatitis and congenital biliary atresia.

Babbitt[3,4] considered an anomalous junction of the pancreaticobiliary ductal system to be an important factor in congenital biliary dilatation.

Komi[12,13] Miyano[16] and Okawa[18] attributed congenital biliary dilatation mainly to an anomalous junction of the pancreaticobiliary ductal system. On the other hand, Kasai et al.[10] stated that of 21 patients with congenital biliary dilatation, 8 had distal obstruction of the dilated choledochus. Katoh[11] found that cystic dilatation of the choledochus was accompopanied by two conditions, i. e., abnormality of the bile duct wall and stenosis of the distal bile duct. Cystic dilatation occurred in young dogs but not in adult dogs. Sptiz[21] succeeded in producing local cystic dilatation of the choledochus after ligation of the distal choledochus of newborn sheep, thinking that the extrahepatic bile duct not surrounded in the liver or spleen is dilated if the obstruction occurs during the fetal or newborn periods.

Miyano et al.[15] induced cyctic dilatation of the extrahepatic bile duct in the same way.

Based on the clinical experience with cystic dilatation in infants in our clinical study on congenital biliary dilatation[17] and the fact that stenosis of the extrahepatic bile duct is one of the causes of the disease, extrahepatic biliary dilatation has been induced in rats after ligation and dissection of the choledochus (severe stenosis) to investigate the nature of the dilatation. Cameron et al.[5] showed that the obstruction of the choledochus was recovered in some rats with 28 days after single ligation of the choledochus and in all rats within 46-84 days after ligation of the choledochus.

In the present study, the ligated choledochus recanalized for 2-11 months after operation in all rats and fusiform type of dilatation (diameter : 1.75 ± 1.07 mm) of the extrahepatic bile duct was seen in 4 of the 9 rats.

No significant differences were noted between the treated and Group I (0.28 ± 0.15 mm).

Irwin[8] first reported a case of congenital choledochal cyst associated with carcinoma. Thereafter, many authors[19),23] reported carcinoma arising in a choledochal cyst.

Paying special attention to this fact, observation was done on 15 rats with cystic dilatation of the extraheparic bile duct for 7-15 months after ligation and dissection of the choledochus, and also on 8 rats with cyst-duodenostomy for 3.5-6 months to investigate carcinoma of the cystic wall. No carcinoma was noted in any Groups.

Dilatation of the bile duct was 7.92 ± 7.05 mm in diameter in Group III-A (weight : less than 100 g) and 6.06 ± 2.03 m in Group III-B (weight : 100 to less than 300 g). The dilatation was more marked in Group III-A young rats than in Group III-B adult rats.

All rats of Group III-C died within 3 weeks after operation. No dilatation of the intrahepatic bile duct was noted in Groups III-A and -B.

In the histological examination, the following findings were observed in Groups III-A and -B : ①Loss of the epothelium of the dilated bile duct wall ; ②Thickening of the bile duct wall, with degeneration and destruction of elastic fiber. These changes in histological findings of the dilated choledochus were very similar to those in patients. The extrahepatic bile duct with cystic dilatation was scarred in Group IV. In the liver, a notable histological change was the proliferation of bile ductuli and B/P ratio was 40 two months after dissection. Changes in parenchymal

cells were not marked in Group III. In Group IV, necrosis and vacuolization of the liver cells and formation of the pseudolobulus and fibrosis were very similar to histological findings of liver cirrhosis in patients.

In biochemistry tests, total bilirubin values increased from early after dissection and were higher in Group IV than in Group III. Total cholesterol values were higher in Gtoup IV than in Group III. It is reasonable that there were no signigicant differences in biochemistry values among Group I, Group II, and Group V-B or between Groups IV and V-A.

Conclusion

In order to elucidate the effect of the choledochal stenosis on congeninital biliary dilatation, 123 rats were used to compare among 5 Groups, (I control, II : single ligation of the choledochus, III : short-term observation after ligation and dissection of the choledochus, IV : long-term observation after ligation and dissection of the choledochus, V : cyst duodenostomy) in macroscopical and histological findings of the extrahepatic bile duct and liver and biochemistry values.

In young rats aged 3～6 weeks, cystic dilatation occured 11 rats out of 39 rats, while older rats these change were not observed except one.

Incidence of the cystic type of the disease may related to the age of the animals at the onset of the stenosis of the choledochus.

Summary

In order to elucidate the effect of the choledochal stenostis on congenital biliary dilation, 123 rats were used and comparisons were made among 5 Groups (Group I : control, Group II : single ligation of the choledochus, Group III : short-term of observation after ligation and dissection of the choledochus, Group IV : loug-term observasion after ligation and dissection of the choledochus, Group V : cyst-duodenostomy).

1) Cystic dilatation of the choledochus was not induced by single ligation.

2) Cystic dilatation localized in the extrahepatic bile duct could be induced in rats by double ligation and dissection of the choledochus.
Cystic dilatation was more pronounced in rats weighing less than 100 g than in those weighing 100 g or more.

3) No cystic dilatation was induced in rats weighing 400 g or more. All these animals died within 3 weeks after ligation and dissection of the choledochus.

4) The intrahepatic bile duct was not dilated in any of the rats with induced cystic dilatation.

5) In the histological examination of the dilated bile duct, loss of the epithelium, thickening of the wall, degeneration and destruction of the elastic fiber were observed. These changes resembled the clinical findings.

6) Histological examination of the liver showed marked proliferation of bile ductuli.

7) Necrosis and vacuolization of the liver cells, formation of pseudolobulus and fibrosis

observed in the liver of Group IV were similar to those in patients with heaptic cirrhosis.

8) No carcinoma was seen in any rats.

9) Cystic dilatation was scarred on observation 7-15 months after ligation and dissection of the choledochus.

10) These results indicate that stenosis of the distal choledochus may be one of the main factors in congenital biliary dilatation, especially cystic type of congenital biliary dilatation.

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和文抄録

先天性胆道拡張症の研究

第2編 先天性胆道拡張症の実験的研究

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中 島 康 夫

先天性胆道拡張症の病因として総胆管末端の狭窄に注目し，ラット123匹を用い，その総胆管を結紮又は離断し下記の5群に分かち検討したところ，以下（①～⑩）の結果を得た．（第Ⅰ群：コントロール群，第Ⅱ群：単純結紮群，第Ⅲ群：結紮離断短期観察群，第Ⅳ群：結紮離断長期観察群，第Ⅴ群：嚢腫十二指腸吻合群）

1) 総胆管の結紮だけではラットに嚢腫状拡張を作成することは出来なかった．

2) 総胆管の結紮離断により，ラットの肝外胆道に限局した嚢腫状拡張を作製し得た．この嚢腫状拡張は，体重100g未満のラットの方が体重100g以上のラットに比して，より大きく拡張した．

3) 体重400g以上のラットは嚢腫状拡張を呈することなく全例，結紮離断後3週間で死亡した．

4) 嚢腫状拡張を示した全例とも，肝内胆管の拡張は認めなかった．

5) 拡張胆管壁の組織学的検討にて，拡張が進行するにつれ，胆管上皮の剝脱，壁の肥厚，弾性線維の破壊等，先天性胆道拡張症例とよく似た所見を呈した．

6) 肝の組織学的所見では，著明な胆管増生が認められた．

7) 結紮離断後長期観察すると，肝は肝細胞壊死，空胞化，偽小葉の形成，線維化の進行等，臨床例の肝硬変によく似た所見を呈した．

8) 結紮離断後長期（術後7ヶ月乃至15ヶ月）観察したラット，嚢腫十二指腸吻合を行ったラットを含めいずれにも癌腫の発生は認めなかった．

9) 嚢腫状拡張は長期（結紮離断後7ヶ月乃至15ヶ月）観察すると瘢痕化した．

10) この実験結果より，本症（特に嚢腫型）の発生には，総胆管壁の未熟な時期において，総胆管末端の狭窄が重要な成因と考えられる．